Pgk1-GFP Cleavage Assay (for Analysis of Non-Selective Autophagy)

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METHOD

To monitor non-selective autophagy (bulk degradation of the cytoplasm), yeast strains expressing the cytoplasmic protein Pgk1 C-terminally fused with GFP are used (we usually tag genomically-encoded Pgk1). For yeast cell culture and induction of non-selective autophagy by starvation or rapamycin treatment, also see the protocols "Cultivation of Yeast Cells and Induction of Autophagy".

1. Culture yeast cells expressing Pgk1-GFP to mid-log phase (OD_{600}=~1.5) at 30°C, and induce autophagy as described in the protocol "Cultivation of Yeast Cells and Induction of Autophagy".

NOTE An *atg* mutant should be included as a control in order to judge whether Pgk1-GFP cleavage occurs in an autophagy-dependent manner under your experimental conditions.

2. Take 1 ml of the culture, and harvest cells by centrifugation at 15,000 *g* for 1 min at 4°C using a centrifuge (MX-105, TOMY). Store the cell pellets at −30°C until all samples are obtained.

NOTE GFP fragments generated by vacuolar degradation of Pgk1-GFP become detected in immunoblotting analysis after nitrogen starvation/rapamycin treatment for ~4 h under our experimental conditions. We usually take samples at 0, 4, and 8 h.

NOTE Cell pellets can be stored at −30°C for a few weeks.

3. Prepare samples, and perform SDS-PAGE and immunoblotting using anti-GFP antibody as described in the protocol "GFP-Atg8 Cleavage Assay". The bands of Pgk1-GFP and GFP fragments will appear near the molecular weight markers of 75 kD and 25 kD, respectively.